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Effects of Testosterone on the Metabolism of Folate Coenzymes in the Rat

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1. The effects of castration and testosterone treatment on enzymic activities involved in folate coenzyme metabolism in the liver and in accessory sex organs of male adult rats were studied. 2. In the liver of castrated rats the concentration of 10-formyltetrahydrofolate (10-HCO-H_4folate) synthetase and tetrahydrofolate (H_4folate) dehydrogenase were significantly decreased whereas that of 5,10-methylenetetrahydrofolate dehydrogenase increased; the treatment with five doses of testosterone caused a return to normal values of these activities. 3. In the prostate of castrated rats a pronounced decrease in H_4folate dehydrogenase, serine hydroxymethyltransferase and 10-HCO-H_4folate synthetase activities was observed. The administration of testosterone restored the enzymic activities to normal values. 4. In the seminal vesicles of castrated rats only 10-HCO-H_4folate synthetase was markedly depressed; testosterone treatment not only restored activity to normal values but raised it to higher than normal values. The slight changes observed in other enzymic activities also returned to normal values with the hormone treatment. 5. These results are discussed in relation to a possible control mechanism of folate metabolism by testosterone.

Researches *in vivo* (Williams-Ashman *et al.*, 1964; Villee & Fujii, 1968) and *in vitro* (Florini & Breuer, 1965; Kochakian, 1967; Pegg & Williams-Ashman, 1968; Liao & Stumpf, 1968) demonstrated that testosterone can increase amino acid incorporation into proteins and activate RNA synthesis in cells. The increase in RNA, mainly of rRNA and mRNA, may be considered the most important biochemical event responsible for the protein-anabolic action attributed to these hormones. To throw light on the mechanism whereby testosterone carries on this stimulating action, we studied (C. Bovina, B. Tolomelli, C. Rovinetti & M. Marchetti, unpublished work) the effect of this hormone on the tissue storage of folate coenzymes, since they are involved in nucleic acid synthesis (Blakley, 1969; Marchetti, 1971). In castrated rats the study showed marked changes in the content and distribution of various folate coenzymes not only in target organs but also in the liver. Testosterone treatment restores the concentrations of these coenzymes to normal, both qualitatively and quantitatively. The influence of testosterone on the conversion of folic acid into its activated forms *in vivo* has been studied by measuring folate derivatives in urine, and concentrations of folate coenzymes in liver, after the injection of folic acid (B. Tolomelli, C. Rovinetti, C. Bovina & M. Marchetti, unpublished work).

The results obtained demonstrated that in castrated rats the amount of folate excreted in the reduced form, and the coenzyme concentrations in liver, are markedly lower than in normal rats. Since treatment of castrated animals with testosterone returns both values to normal, it is supposed that the hormone is somehow involved in folate coenzyme metabolism. To confirm this hypothesis more directly, some enzymic activities involved in the synthesis of these compounds were studied. In particular H_4folate* dehydrogenase (EC 1.5.1.3), 10-HCO-H_4folate synthetase (EC 6.3.4.3), 5,10-CH_2-H_4folate dehydrogenase (EC 1.5.1.5) and serine hydroxymethyltransferase (EC 2.1.2.1) were determined in accessory sex organs and in the liver of castrated rats as well as in castrated rats treated with testosterone.

Experimental

Materials

All chemicals, enzymes and coenzymes (except H_2folate and H_4folate) were obtained commercially. H_2folate was prepared by reduction of the folate with dithionite (Futterman, 1957). H_4folate was prepared by catalytic hydrogenation of folate over platinum oxide in acetic acid (O'Dell *et al.*, 1947).

Animals

Male albino rats (Wistar), 15 weeks old, 350–400g in weight, divided into four groups, were used. The animals of groups 3 and 4 were castrated via the scrotal route under ether anaesthesia. After 4 weeks

* Abbreviations: H_2folate, dihydrofolate; H_4folate, tetrahydrofolate; 5,10-CH_2-H_4folate, 5,10-methylenetetrahydrofolate; 10-HCO-H_4folate, 10-formyltetrahydrofolate; 5,10-CH=H_4folate, N^5N^{10}-methylidynetetrahydrofolate.

the rats of groups 2 and 4 were injected subcutaneously with five doses of testosterone propionate (1 mg in 0.2 ml of sesame oil/100 g body wt.) every other day for 10 days. The rats of groups 1 and 3 were injected with the same volume of sesame oil. The rats were fed on a stock diet with no restriction of food intake throughout the experiment. They were killed by cervical fracture 48 h after the last injection and liver, ventral prostate and seminal vesicles were quickly removed and placed in ice-cold water.

Analytical methods

For assaying H_4folate dehydrogenase the tissues were homogenized in 4 vol. of 0.01 M-tris–HCl buffer, pH 7.0, and centrifuged at 20000$g_{av.}$ for 10 min at 4°C. The enzyme was determined in the supernatant by measuring the decrease in E_{340} caused by the conversion of NADPH into $NADP^+$ and of H_2folate into H_4folate (Mathews *et al.*, 1963).

For assay of the other enzyme activities, the tissues were homogenized in 9 vol. of 0.05 M-tris–HCl buffer, pH 7.5, and centrifuged at 10000$g_{av.}$ for 30 min at 4°C. Serine hydroxymethyltransferase was assayed in the supernatant by measuring colorimetrically both the free and the bound formaldehyde in 5,10-CH_2-H_4-folate with the acetylacetone reagent (Scrimgeour & Huennekens, 1962). 5,10-CH_2-H_4folate dehydrogenase was assayed by determining spectrophotometrically at 355 nm the 5,10-CH=H_4folate formed in the system (Scrimgeour & Huennekens, 1963). 10-HCO-H_4folate synthetase was assayed in the supernatant after partial purification with protamine sulphate and solid ammonium sulphate, by measuring the 5,10-CH=H_4folate formed in the reaction mixture (Rabinowitz & Pricer, 1963).

Protein was determined by the colorimetric method of Lowry *et al.* (1951), with crystalline bovine plasma albumin as the standard.

Results

The results in Table 1 relating to liver enzymes show that in castrated rats, as compared with normal animals, there is a significant decrease in 10-HCO-H_4-folate synthetase ($P<0.05$) and H_4folate dehydrogenase ($P<0.001$) activities, and an increase in 5,10-CH_2-H_4folate dehydrogenase ($P<0.001$); no variation was observed in serine hydroxymethyltransferase activity. The treatment of normal rats with testosterone does not cause significant change, whereas the treatment of castrated animals completely returns 10-HCO-H_4folate synthetase activity to normal values and, only partially, 5,10-CH_2-H_4-folate dehydrogenase and H_4folate dehydrogenase activities.

The results in Table 2 relating to the enzymes of the prostate show in castrated rats a decrease of

Table 1. *Effects of castration and testosterone treatment on enzymic activities catalysing the metabolism of folate coenzymes in rat liver*

Methods of treatment of the rats and methods for measuring the enzymic activities are described in the Experimental section. Values are expressed as means ± S.E.M. of observations on the numbers of animals given in parentheses. Significance of differences from values for normal animals: ***$P<0.05$; **$P<0.01$; *$P<0.001$.

Group no.	Experimental animals	H_4folate dehydrogenase (nmol of H_2folate reduced/min per mg of protein)	Serine hydroxymethyltransferase (nmol of HCHO utilized/20 min per mg of protein)	10-HCO-H_4folate synthetase (nmol of 5,10-CH=H_4folate formed/20 min per mg of protein)	5,10-CH_2-H_4folate dehydrogenase (nmol of 5,10-CH=H_4folate formed/20 min per mg of protein)
1	Normal	4.78 ± 0.16 (5)	632 ± 25 (5)	1623 ± 145 (5)	181 ± 15 (5)
2	Normal + testosterone	4.96 ± 0.16 (4)	689 ± 43 (4)	1548 ± 74 (4)	214 ± 6 (4)
3	Castrated	4.15 ± 0.12 (15)*	613 ± 24 (15)	1172 ± 115 (7)***	297 ± 15 (19)*
4	Castrated + testosterone	4.35 ± 0.21 (9)	623 ± 17 (6)	1426 ± 55 (6)	249 ± 17 (6)**

Table 2. *Effects of castration and testosterone treatment on enzymic activities catalysing the metabolism of folate coenzymes in rat ventral prostate*

Methods of treatment of the rats and methods for measuring the enzymic activities are described in the Experimental section. Values are expressed as means ± S.E.M. of observations on the numbers of animals given in parentheses. Significance of differences from values for normal animals: ***$P<0.05$; **$P<0.01$; *$P<0.001$.

Group no.	Experimental animals	Serine hydroxymethyltransferase (nmol of HCHO utilized/20min per mg of protein)	10-HCO-H_4folate synthetase (nmol of 5,10-CH=H_4folate formed/20min per mg of protein)	5,10-CH_2-H_4folate dehydrogenase (nmol of 5,10-CH=H_4folate formed/20min per mg of protein)
1	Normal	79 ± 5.4 (10)	445 ± 45 (8)	40.9 ± 2.5 (10)
2	Normal + testosterone	85 ± 4.2 (6)	380 ± 18 (4)	37.2 ± 1.7 (8)
3	Castrated	10 ± 1.3 (12)*	18 ± 5 (12)*	15.4 ± 1.3 (12)*
4	Castrated + testosterone	101 ± 5.1 (13)**	329 ± 22 (9)***	59.6 ± 3.5 (13)*

Table 3. *Effects of castration and testosterone treatment on enzymic activities catalysing the metabolism of folate coenzymes in rat seminal vesicles*

Methods of treatment of the rats and methods for measuring the enzymic activities are described in the Experimental section. Values are expressed as means ± S.E.M. of observations on the numbers of animals given in parentheses. Significance of differences from values for normal animals: **$P<0.01$; *$P<0.001$.

Group no.	Experimental animals	Serine hydroxymethyltransferase (nmol of HCHO utilized/20min per mg of protein)	10-HCO-H_4folate synthetase (nmol of 5,10-CH=H_4folate formed/20min per mg of protein)	5,10-CH_2-H_4folate dehydrogenase (nmol of 5,10-CH=H_4folate formed/20min per mg of protein)
1	Normal	105 ± 8.9 (6)	16.9 ± 2.71 (6)	4.7 ± 0.70 (10)
2	Normal + testosterone	97 ± 10.0 (6)	17.2 ± 0.58 (6)	3.9 ± 0.15 (4)
3	Castrated	72 ± 4.5 (17)*	2.5 ± 0.17 (19)*	43.6 ± 2.01 (28)*
4	Castrated + testosterone	93 ± 4.6 (10)	27.3 ± 0.41 (5)**	6.0 ± 0.85 (13)

5,10-CH_2-H_4folate dehydrogenase ($P<0.001$), serine hydroxymethyltransferase ($P<0.001$) and 10-HCO-H_4folate synthetase activities ($P<0.001$). Treatment with testosterone does not cause modification in normal rats, whereas in castrated rats it completely restores serine hydroxymethyltransferase to normal values and, partially restores 10-HCO-H_4folate synthetase activities; it increases 5,10-CH_2-H_4folate dehydrogenase activity. Because of the low activity of H_4folate dehydrogenase in prostate, the results have to be considered as only indicative, so no value has been given in the table. However, this enzyme seems to behave in the same way as the other enzymes.

The results in Table 3, relating to the enzymic activities in seminal vesicles, show a marked decrease in 10-HCO-H_4folate synthetase ($P<0.001$) and in serine hydroxymethyltransferase ($P<0.001$) activities in castrated rats and a significant increase in 5,10-CH_2-H_4folate dehydrogenase activity ($P<0.001$).

The treatment of castrated rats with testosterone completely restores 5,10-CH_2-H_4folate dehydrogenase and serine hydroxymethyltransferase activities to normal, whereas it significantly increases 10-HCO-H_4folate synthetase activity. Also, in the seminal vesicles of castrated animals H_4folate dehydrogenase decreases, but its activity is too low for an exact quantitative evaluation.

Discussion

From the results reported in the present paper it is evident that castration can effect severe alterations in enzymic activities catalysing the principal steps of folate coenzyme metabolism. The decreased activity of these enzymes and, in particular, of H_4folate dehydrogenase and 10-HCO-H_4folate synthetase, could explain the decrease in reduced and formylated folate forms observed in the liver and in accessory sex organs of castrated animals. In fact, the more severe the alterations of enzymic activities in an organ, the greater the change of its content of coenzymes. A connexion between castration and alteration in coenzyme content seems to be further supported by the observation that testosterone treatment of castrated rats may restore their enzymic activities to normal and at the same time restore normal coenzyme concentrations. These results suggest that testosterone can affect folate metabolism and especially the synthesis of its coenzymic forms not only in accessory sex organs, which are recognized target organs, but also in the liver. The fact that testosterone may affect the liver, which is not a specific target organ, need cause no surprise when one considers the central role played by this organ in the metabolism of folic acids. It has already been observed that sex hormones can cause a great variety of biological effects on the liver (Song & Kappas, 1968).

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